features vectors and cells containing the claimed nucleic acids.

Summary of the Office Action

Claims 10, 12-14, and 24-25 stand rejected under 35 U.S.C. § 103, and claims 24 and 25 stand further rejected under 35 U.S.C. § 112, first and second paragraphs.

Amendments

The specification and claims 24 and 25 have been amended to correct minor errors and to bring the claims into conformance with the sequence listing requirements. A new sequence listing is submitted herewith that reflects the addition of SEQ ID NO: 17 to claim 25. No new matter has been added by these amendments.

Rejection under 35 U.S.C. § 112, first paragraph

Claim 25 stands rejected, under 35 U.S.C. § 112, first paragraph, based on the assertion that applicants' specification does not contain a written description of a nucleic acid encoding a P-selectin ligand polypeptide which includes Ile135 through Ser225 of the CD43 sequence. In particular, the Office Action requests clear support for the Ile135 limitation.

In response, applicants draw the Examiner's attention to the specification at page 12, lines 9-19, where it states:

CD34, **CD43**, and GlyCAM-1 mucins were prepared for addition of the PSGL-1 amino-terminal domain by appending an EcoRI site to either the mature amino terminus (CD34 or GlyCAM-1), **or to the beginning of a region of threonine/proline-rich repeats (CD43)**. As above, the EcoRI site was in the frame glutamic acid phenylalanine (frame 1). The CD34 sequence began at residue F30 of the precursor, the Gly-CAM-1 at precursor L19, **and the CD43 at precursor I135.** To each of these was appended the flu-tagged PSGL-1 domain terminating in EcoRI as above. The amino terminus and repeat elements of PSGL-1 were appended to the membrane proximal, transmembrane, and intracellular domains of CD43 through an EcoRI site in the glutamic acid phenylalanine frame positioned immediately upstream of the sequences S225 of the CD43 precursor.

Thus, applicants' specification provides clear support for the portion of CD43 currently claimed, and, in particular, provides clear support for a CD43 fragment beginning at amino acid I135. In view of this description in the specification, the § 112, first paragraph rejection may be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 24 and 25 also stand rejected, under 35 U.S.C. § 112, second paragraph, as containing claim terms that are unclear or indefinite.

Specifically, claim 24 stands rejected based on the assertion that the claim term "consists essentially of" is unclear, particularly in the context of a recited sequence identification number. This claim has been amended to read "consists of," and the rejection of claim 24 may be withdrawn.

Claim 25 stands rejected on the basis that the terms I135 and S225 should be

expanded to reflect their standard three-letter amino acid designations. This claim has been amended as suggested by the Examiner, and the rejection of claim 25 may also be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 10, 12-14, and 24-25 stand further rejected, under 35 U.S.C. § 103, for obviousness over Larsen et al. (U.S. Patent No. 5.843,707; "Larsen"), Sasaki et al. (*J. Biol. Chem.* 269:14730, 1994; "Sasaki"), or Sako et al. (*Cell* 75:1179-1186, 1993; "Sako"), in view of Aruffo et al. (*Cell* 67:35-44, 1991; "Aruffo") and Lowe (U.S. Patent No. 5,595,900).

These claims recite nucleic acids encoding polypeptides that are synthetic P-selectin ligands and that contain sialyl Le^x addition sites and tyrosine sulfation sites, wherein at least one of these sites is located at an amino acid position that is different from its position in a naturally-occurring P-selectin ligand. The creation of applicants' synthetic P-selectin ligands was made possible by their surprising discovery that the positioning of the sialyl Le^x and tyrosine sulfation sites could be changed from their naturally-occurring positions and the ligand would nonetheless maintain its biological activity. Prior to this inventive discovery, it was generally believed that these sites were fixed within the molecule so that the ligand was forced to adopt a specific three-dimensional structure necessary for ligand function and receptor interaction. This was

particularly true given the necessity for two distinct P-selectin recognition domains; unlike E-selectin, for example, which apparently relies on only the glycan sialyl Le^x for receptor-ligand interactions, the P-selectin receptor needs to interact with two different ligand domains, highlighting the presumed importance of structural integrity to the ligand's proper function. Consistent with this general belief about the importance of three-dimensional structural constraints to P-selectin ligand function, nothing in the art suggested flexible positioning of either the sialyl Le^x or tyrosine sulfation sites, or that synthetic P-selectin ligands having non-naturally-occurring sites should, or could, be made.

Looking, for example, to the references cited in this case, Larsen teaches a number of P-selectin ligands. These include the full-length naturally-occurring ligand, as well as mature ligands lacking signal sequences, soluble forms of the ligand, and P-selectin fragments possessing ligand activity. Despite the number of ligand alterations discussed by Larsen, never once does Larsen suggest that the sialyl Le^x or sulfated tyrosine sites could be moved from their naturally-occurring positions. This is particularly striking in light of the fact that Larsen states an intent to use the P-selectin ligand for therapeutic purposes (col. 15-17), a use in which it would be extremely valuable to replace the large 402 amino acid P-selectin ligand with a synthetic ligand having similar biological properties. Nevertheless, Larsen restricts any P-selectin ligand alterations to those in which the sialyl Le^x and tyrosine sulfation sites remain intact and identical in position to

those sites in the naturally-occurring protein.

Similarly, Sako, which discusses the cloning of the P-selectin glycoprotein ligand, PSGL-1, contrasts this ligand with the E-selectin ligand, which as mentioned above relies only on sialyl Le^x moieties for receptor interactions. Sako states that P-selectin function, unlike E-selectin function, requires the involvement of "the polypeptide" (rather than just the post-translationally added glycan moieties). Specifically, Sako states:

> Like L-selectin, PSGL-1 most likely bears the SLe^x moiety that can mediate E-selectin binding. Thus, simple presentation of SLe^x may be sufficient to mediate E-selectin binding, regardless of the "scaffolding" (i.e., lipid or protein) employed for its presentation. . . . However, our results clearly demonstrate that **the polypeptide is critical in defining PSGL-1 as a ligand for P-selectin**.

Thus, Sako, like Larsen, indicates that the polypeptide itself is important — in fact, critical — to ligand function. Sako therefore in no way indicates that the P-selectin polypeptide may be changed, and if anything suggests that it can not.

The remaining references do not directly bear on this issue, being even further from applicants' invention. Aruffo discusses only sulfatides, which are lipids (specifically, heterogeneous 3-sulfated galactosyl ceramides), as ligands of CD62/P-selectin. While Aruffo does demonstrate that, in the context of lipid ligands, sulfation is important, this is a far cry from suggesting applicants' synthetic P-selectin polypeptide ligands. Aruffo, in fact, does not teach a single P-selectin polypeptide ligand or, for that matter, even suggest that one exists.

And Sasaki and Lowe are limited to disclosures of particular glycosyltransferase enzymes and their coding sequences. Sasaki focuses on an enzyme that adds sialyl Le^x moieties and its possible role in E-selectin function, and Lowe discloses a number of different glycosyltransferases and a general method for cloning post-translational modification enzymes without "the need to first isolate the protein products of these genes." Contrary to the assertion in the Office Action, Lowe does not provide "nucleic acids that encode for glycosylation and sulfation sites in glycoproteins of interest." Rather, Lowe suggests that the general cloning method provided in the specification could be applied to the possible isolation of a long list of other enzymes that modify proteins, including sulfation enzymes. Lowe does not disclose or suggest a single sulfation enzyme. Nor does Lowe suggest, anywhere in the specification, the use of a disclosed enzyme for the production of any type of P-selectin ligand.

In short, the prior art does not support an obviousness rejection of applicants' nucleic acids encoding synthetic P-selectin ligands. Indeed, the current rejection turns on the assertion that:

> As pointed out previously, it was known at the time the invention was made that both sialyl and sulfation sites and structures contributed to P-selectin binding. Given the importance of both sialyl and sulfation moieties to the affinity and avidity of P-selectin-mediated binding, it would have been obvious to one of ordinary skill in the art at the time the invention was made to provide such moieties to modified/altered P-selectin ligands. It is noted that Larsen et al. teach nucleic acids encoding various modified/altered P-selectin ligands, including fragments and fusion proteins thereof for various uses (see columns 8-18). The

prior art also provided for nucleic acids that encode for glycosylation and sulfation sites in glycoproteins of interest (for example, see Lowe et al., column 14, paragraph 2). Therefore, the ordinary artisan would have been motivated and would have expected the provision for sialyl and sulfation sites at non-naturally occurring sites in the generation of various modified/altered P-selectin ligands either to provide for or to increase affinity/avidity of such molecules.

Applicants respectfully disagree. As noted above, Larsen does not describe a single P-selectin molecule with a sialyl Le^x or sulfation site in a non-naturally occurring position. Indeed, despite the large number of potential proteins mentioned by Larsen, all of Larsen's modified ligands are shortened versions of the naturally-occurring protein, each perfectly preserving the spacing between the sialyl Le^x and tyrosine sulfation sites.

In addition, while Larsen does describe molecules in which the sialyl or tyrosine sulfation sites are altered, sometimes in very small ways, these alterations all result in greatly diminished ligand activity. For example, as shown in Figure 12, deletion of the sialyl Le^x site destroyed P-selectin binding in the Larsen assay. And, as shown in Figures 16 and 21, even a single amino acid substitution in the naturally-occurring tyrosine sulfation site reduced ligand activity, and the replacement of three tyrosine residues resulted in complete destruction of ligand function. These results would certainly not motivate one skilled in the art to modify either the sialyl Le^x or sulfation sites in a P-selectin ligand in any way. Quite to the contrary, the results of Larsen, when viewed objectively and in the absence of applicants' disclosure, would discourage changes to the P-selectin ligand that might be expected to disrupt the function of the sialyl Le^x or

tyrosine sulfation sites. And nothing in Lowe's disclosure of particular glycosyltransferases or enzyme cloning techniques — or in any other reference cited in current Office Action — cures this deficiency.

In light of the above arguments, applicants request reconsideration on the obviousness rejection in this case and withdrawal of the current § 103 rejection of claims 10, 12-14, and 24-25.

Conclusion

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for three months, to and including February 29, 2000. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 29 February 2000

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